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BIOCHEMICAL AND MICROBIOLOGICAL STUDIES ON CLAM *VILLORITA CYPRINOIDES*

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ABSTRACT

Clam muscle is a rich source of protein with all essential amino acids, fat, glycogen and minerals with low protein content (6 to 11%) and with high carbohydrate compared to fish. Fractionation of clam muscle protein reveals higher percentage of sarcoplasmic (34.5%) than myofibrillar proteins (30.2%). Lysine and leucine predominate the free amino acid pool. Pathogenic organisms like *Coliforms* and *Faecal streptococci* have been isolated. Composition of clam shell liquor is reported.

INTRODUCTION

CLAMS belong to one of the most important varieties of shellfishes. They are harvested abundantly in Canada, United States, Chile, China, Japan and Korea. In India nine species contribute to the fisheries of commercial importance. Black clam *Villorita cyprinoides* is available in Cochin and several other places in the South India. They are available throughout the year and harvested for the lime and cement industries which turns out a large quantity of meat as a byproduct.

Spoilage pattern of clam muscle during cold storage and loss of nutrients during canning was studied in detail (Chinnamma, 1974; Chinnamma and Gopakumar, 1988). Investigations carried out by Sandholzer and Arcisz (1946) showed that coliforms, *Bacillus* and *Pseudomonas* occurred in clam meat and occurrence of paralytic shellfish poisoning on the west coast of United States was summarised by Pivnick (1951). The shellfish can be made safe for human consumption by a process of self purification has been demonstrated by Connell (1980).

The clam freezing and canning industry has shown a phenomenal growth in India since 1980, the country today is one of the leading exporters of clam meat as frozen, canned and pickled products to different countries.

A critical study from the nutritional point of view does not appear to have been made even after the existence of processing industries based on clam meat. Present communication summarises the results of investigations on biochemical composition, protein fractions, amino acid pattern, composition of clam shell liquor and microbial count.

MATERIALS AND METHODS

Clam *Villorita cyprinoides* 2.0 - 3.5 cm height collected from Cochin Backwater were brought to the laboratory in live condition. After thorough washing the shell was opened with a stainless steel scalpel and muscle was transferred into sterile dishes for microbiological analysis using sterile scissors. For biochemical studies the muscle scooped out was strained through stainless steel strainer kept at 0°C for two minutes collecting the free liquid. Meat weight and volume of drained liquor was noted. The intestinal part was removed and the muscle

was minced thoroughly. The minced meat and drained liquor were subjected to detailed analysis. Three series of experiments using 100 g of comminuted meat for each sampling were conducted during January to April for protein fractions, free amino acid composition and shell liquor constituents. The biochemical composition and microbial flora, the range of values given are from ten series of experiments conducted during different seasons of the year and from different localities around Cochin.

Moisture, protein, ash and lipids were estimated according to the AOAC (1975) procedure. Protein fractionation was carried out by the preferential solubility techniques of King (1966) and Paul *et al.* (1966) as described by Chinnamma and Gopakumar (1987). Water soluble nitrogen and non-protein nitrogen determinations were made according to the method described by Chinnamma and Gopakumar (1987). Free alpha amino nitrogen was estimated by the method of Pope and Stevens (1939). Extraction of free amino acids was done according to Jones (1959) and identified by the microbiological assay method of Schockman (1963). Glycogen was estimated by the method of Van de Kleiy (1951), pentose by the method of Mejbaum (1939), phosphorus by the procedure of Fiske Subbarow (1925).

Microbiological examination of samples for standard plate count, coliforms and faecal streptococci were done according to FDA, Bacteriological analytical manual (1978).

Results and Discussion

Water, protein, fat and carbohydrates are the main constituents of fish and shellfish with non-protein nitrogenous constituents and salts in small measures. For clams yield varied from 8.9 to 13% (Chinnamma *et al.*, 1986). Chemical composition and microbial count of clam muscle are presented in Table 1. Moisture content was higher and protein slightly lower with medium fat content and high proportion of sand was detected, Standard plate count, coliforms and faecal streptococci were on the higher side.

Table 1. *Proximate chemical composition and microbial count of clam meat* (Villorita *sp.*)

Moisture (%)	79.5	–	84.6
Protein (TN × 6.25) (%)	6.6	–	11.0
Water extractable nitrogen (mg%)	420.0	–	564.0
Non-protein nitrogen (mg%)	191.0	–	428.0
Free alpha amino nitrogen (mg%)	32.3	–	205.0
Glycogen (%)	1.4	–	7.8
Phosphorus (inorganic) (mg%)	22.0	–	32.0
Pentose (mg%)	79.0	–	187.0
Fat (%)	1.1	–	2.1
Ash (Dry weight basis) (%)	9.8	–	11.2
Acid insoluble ash (sand) (DWB) (%)	1.9	–	3.54
Standard plate count/g	3.2×10^5	–	8.4×10^5
Coliforms/g	103.0	–	172.0
Faecal streptococci/g	120.0	–	154.0

According to Mannan *et al.* (1961), important factors which influence the composition of any particular species of fish are the abundance of food, spawning cycle, spawning migration and age. The wide variations observed in the values are due to seasonal changes. Galtsoff (1964), Chinnamma and James (1971) observed variation in composition according to season in American oyster and crab *Scylla serrata*. The different fractions of clam muscle protein (Table 2) revealed higher percentage of sacroplasmic (34.54%) than myofibrillar protein (30.2%). Non-protein nitrogen was found to be high (25.1% of TN) and denatured protein content showed high value of 34.81%, probably due to the denaturation of the myofibrillar protein during successive extractions. Stroma protein content was very low. This agrees with the pattern observed by several workers (Maruyama and Suzuki, 1969) and Paul et al. (1966).

TABLE 2. *Protein fractions of clam muscle* (Villorita *sp.*)

Total nitrogen (g%)	1.78
Protein nitrogen (g%)	1.33
Non-protein nitrogen (% of TN)*	25.13
Sarcoplasmic protein (% of PN)**	34.54
Myofibrillar protein (% of PN)	30.20
Denatured protein (% of PN)	34.81
Stroma protein or connective tissue (% of PN)	0.44

* TN — Total nitrogen
** PN — Protein nitrogen

Table 3 gives the free amino acid pattern of clam muscle. Fourteen amino acids were identified and estimated. Lysine (17.7%) and leucine (14.5%) constitute the major amino acids in clam muscle, with phenylalanine, histidine, prolein, tyrosine, glycine, glutamic acid, serine, methionine and isoleucine in moderate quantities in decreasing order and cystine, valine and tryptophan in trace quantities.

TABLE 3. *Free amino acids in clam muscle* (Villorita *sp.*)

Amino acids	Quantity % of total free amino acids
Phenylalanine	11.82
Glycine	6.83
Cystine	0.083
Tyrosine	8.85
Histidine	11.83
Valine	0.42
Lysine	17.75
Methionine	3.55
Glutamic acid	6.72
Isoleucine	2.65
Leucine	14.58
Tryptophan	0.053
Serine	3.90
Proline	11.50

It is a characteristic feature of all shellfishes to retain some liquid in their body to survive unfamiliar conditions caused by floods or by the temporary presence of toxic or irritating substances in the water. Various metabolic products accumulate in the shell liquor.

The analytical values of nutrients based on 100 gm muscle weight in the liquid collected in the clam shell are given in Table 4. As can be seen from Table 4, 241 mg nitrogen 11.5 mg non-protein nitrogen, 30 mg glycogen 1.2 mg phosphorus (inorganic) and 1.9 mg pentose are present in the clam juice.

TABLE 4. *Composition of clam juice*

Yield on the basis of whole weight	8.9 - 13.0%
Juice volume	29.6 ml per 100 g muscle
Total nitrogen (mg%)	241
Water soluble nitrogen (mg%)	117
Non-protein nitrogen (mg%)	11.5
Free alpha amino nitrogen (mg%)	1.7
Glycogen (mg%)	30.3
Phosphorus (inorganic) (mg%)	1.2
Pentose (mg%)	1.9

Values presented are on the basis of the muscle weight

Processing industries based on clam meat has immense possibility to develop in future, because of the abundant availability of the nutritious raw materials.

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